EVALUATION OF NUTRITIVE VALUE AND *IN VITRO* METHANE PRODUCTION OF FEEDSTUFFS FROM AGRICULTURAL AND FOOD INDUSTRY BY-PRODUCTS

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ABSTRACT

The aim of this research was to evaluate the nutrient degradability, *in vitro* methane (CH_4) production of several agricultural and food industry by-products in relation to their chemical composition. Twenty-one samples of 7 feedstuffs from agricultural and food industry by-products consisted of corn straw, potato straw, rice straw, cocoa pod, sago waste, rice bran, soybean curd residue were evaluated by an *in vitro* gas production and nutrient degradability. The feedstuffs varied greatly in their crude protein (CP), neutral detergent fiber (NDF) and non-fiber carbohydrate (NFC) contents. Crude protein ranged from 1.5 to 21.8%, NDF from 31.6 to 71.1% and NFC from 1.5 to 50.8%. Among the seven feedstuffs, soybean curd residue had the highest CP content, on the other hand it had the lowest NDF content. Dry matter (DM) and organic matter (OM) degradability were highest ($P<0.01$) in soybean curd residue among the feedstuffs. The CH_4 production was significantly higher ($P<0.01$) in rice straw, cocoa pod and corn straw as compared to sago waste. There was a strong positive correlation (r = 0.60; $P<0.01$) between NDF concentration and CH_4 production. However, the total gas productions was negatively correlated (r = -0.75; $P<0.01$) with NDF content. The CH_4 production of feedstuff is influenced by NDF content.

Keywods: Agricultural, by-product, Fermentation, In vitro, Methane.

INTRODUCTION

Feed availability as quality, quantity or continuity is important factor to support development of animal production system. In the tropical country such as Indonesia, grass production is very much during wet season, but during dry season its production is limited, thus most of farmers have problem to serve forage for ruminants. Leng (1990) reported that most of farmers in tropical and subtropical countries used agricultural by-product as major component of diet for large ruminant for considerable part of or throughout the year.

Generally, agricultural by-products have low quality because they have high crude fiber content but low crude protein and digestibility value. However, ruminants have ability to convert low-quality forages become useful products as meat and milk for human. Methane (CH_4) produced during digestion in the rumen represents energy loss to the host animal and contributes to global warming. Kurihara *et al*. (1999) stated that CH_4 production in cattle fed on tropical forage diet was higher than those fed on temperate forage diet, due to grass in the tropics containing high crude fiber and lignin but low non-structural carbohydrate than grass in the subtropics.

According to Estermann *et al*. (2002) there was a strong relationship between CH_4 production and NDF intake and NDF digested. Santoso *et al*. (2007) also observed a strong relationship between CH_4 production (g/day) and NDF digested (g/day) (r = 0.88). In other study, Moss *et al*. (2002) concluded that CH_4 production has positive correlation with NDF content (R^2=79%).

The CH_4 production has a negative correlation with energy utilization in ruminants. Energy lost as CH_4 from mature cattle ranges from 2 to 12% of gross energy intake. The range in CH_4 emissions is due to mainly to the level of feed intake and the composition of the diet (Johnson and Johnson, 1995).

Measurement of CH_4 in feedstuffs from agricultural and food industry by-products as sole feed or concentrate component in Indonesia are limited. Based on above reasons, a study is needed to evaluate nutrient degradability i.e. dry matter (DM) and organic matter (OM), fermentation characteristics, CH_4 production in feedstuffs from agricultural and food industry by-product.

MATERIALS AND METHODS

Preparation of Feedstuffs

Twenty-one samples of 7 feedstuffs from agricultural and food industry by-products consisted of corn straw, potato straw, rice straw, cocoa pod, sago waste, rice bran, soybean curd residue were used in *in vitro* nutrient degradability and gas production. Each feedstuff was collected from district of Manokwari, Prafi and Oransbari. Samples which contained less than 20% dry matter were oven dried at 60 ºC for 48 h, then ground to pass a 1 mm sieve in a Wiley mill.

Donor Animal

Two ruminally fistulated Ongole crossbreed cattle were used as rumen liquor donor. Animals were fed twice daily (08:00 and 16:00 h) at maintenance level of DM intake with a basal diet consisting of elephant grass and concentrate (70 : 30, on DM basis).

In vitro Gas Production Test

Gas production measurement was conducted according to method of Menke and Steingass (1988), as previously described by Santoso and Hariadi (2008). Samples (300 ± 10 mg) were weighed into 100 ml glass syringes (Model Fortuna, Häberle Labortechnik, Germany). Buferred mineral solution was prepared just before experiment and placed in a water bath under continous flushing with CO_2. About 30 ml of rumen liquor-buffer mixtures in 1 : 2 ratio was dispensed into syringes. The syringes were incubated in a water bath at 39 °C for 48 h. The volume of gas released from each syringe was recorded at 2, 4, 6, 12, 24 and 48 h of incubation, whereas sample of CH_4 was collected at 12 and 48 h of incubation. Methane was determined using a chromatograph gas (Hitachi 263-50). At the end of the incubation period, sub sample of rumen liquor-buffer mixtures were analyzed for volatile fatty acids (VFA) and NH_3-N concentrations as described in detail by Santoso and Hariadi (2008).

In vitro Degradability Test

In vitro degradability was determined following the first phase of Tilley and Terry (1963), as used by Santoso and Hariadi (2008). Twenty five milliliter of rumen liquor-buffer mixtures in a 1 : 4 (v/v) ratio were dispensed in 100 mL glass tubes containing 250 mg of dry sample. After gassing CO_2 in the tube, corks were tightly placed over the tubes and were incubated in a water bath at 39 °C for 48 h. After 48 h of incubation, the contents were filtered through pre-weighed Gooch crucibles and dried at 105 °C for 24 h. The percent loss in weight was determined and presented as DM degradability. The residue left was ashed at 550 °C for determination of OM degradability.

Sample Analyses

The DM, ash, crude protein and ether extract (EE) of samples were analyzed according to method described by Harris (1970), whereas NDF content analysis was according to Van Soest *et al*. (1991). The NFC concentration was calculated as OM – (CP + NDF + EE) (Kurihara *et al.*, 1999).

Experimental Design and Statistical Analysis

The experiment was arranged in completely randomized design consisted of 7 feedstuffs with 3 replications. Data were analyzed using analysis of variance using general linear model procedure of SAS. Treatment means were separated by applying Duncan's multiple range test with a probability level of P=0.01. Pearson correlation was used to establish the correlations among variables.

RESULTS AND DISCUSSION

Chemical composition of feedstuffs used in this study is presented in Table 1. Soybean curd waste had the highest CP content (21.8 4.6%), and sago waste was observed to be the lowest (1.4 ± 0.2%). The CP content of soybean in this research was similar to value of 23.6% as reported by Wina *et al*. (2008),

Table 1. Chemical Composition of Feedstuffs (%) from Agricultural and Food Industry By-product

Feedstuffs	DM	OM	CP	EE	NDF	NFC
			---------------------% DM---------------------			
Corn straw	24.6	91.2	11.9	8.0	63.0	8.3
Potato straw	14.2	88.9	15.6	11.1	42.3	19.9
Rice straw	82.3	81.1	6.8	1.7	71.1	1.5
Cocoa pod	16.1	90.3	6.8	5.1	47.7	30.7
Sago waste	6.8	91.4	1.5	0.8	38.3	50.8
Rice bran	87.1	88.7	9.4	9.9	36.6	32.8
Soybean curd residue	12.1	96.2	21.8	8.0	31.6	34.8

DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; NFC: non-fiber carbohydrate; SE: standard of error

however Kondo *et al.* (1996) reported that DM and CP contents of soybean curd waste were 17.9% and 26%, respectively. The CP concentration of potato straw and corn straw in this study was higher than CP content of potato straw (11.3%) and corn straw (7.4%) as reported by Tangendjaja and Gunawan (1988). Concentration of CP of sago waste in this study was comparable to value of 0.98% as reported in earlier study (Kumoro *et al.*, 2008). In addition, CP and EE contents of rice straw in this study were similar to result of Utomo (2004) that 5.72% and 1.50%, respectively. The NDF content of rice straw was lower than result of Liu *et al.* (2002) with NDF concentration 75%. The different result of this study as compared to other study could be due to different of planting location, harvest time and variety. Crowder and Chheda (1982) suggested that the minimum level of feed CP for fermentation and degradation in the rumen is 7%. However, a study with tropical grass Minson (1990) noted that rumen bacteria need 6% of CP for normal activity. Based on Table 1, using sago waste as ruminant's feed required other feedstuff which has high CP. Sago waste contains the lowest EE content but the highest NFC. Rice straw had the highest NDF content and the concentration was similar with value of 72.5% as reported by Wang *et al.* (2006). High NDF content in rice straw (71.1%) caused low in NFC content. The CP content of rice bran in this study was similar with result of Prasetyono *et al.* (2007). Tangendjaja (1990) reported that CP content of rice bran with some milling levels ranged from 14.23 to 15.10%.

The DM and OM degradability of soybean curd waste was higher (P<0.01) than other feedstuffs. In addition, DM degradability on corn straw, sago waste, potato straw and rice bran were higher than cocoa pod and rice straw. The higher DM degradability in soybean curd waste could be due to high CP content, possibly resulting in increased activity and microbe population during fermentation. This condition was followed by increasing in DM and OM degradability. Even though sago waste contained the lowest CP, NDF content was also low thus DM degradability was not too low. In contrast, rice straw had the highest NDF content (71.1%), causing the lowest DM and OM degradability. Tangendjaja (1990) stated that the *in vitro* DM digestibility of rice straw was 35.4%. Moreover, in general the *in vitro* DM digestibility this feedstuff was below 40%. The DM degradability of corn straw in this study was higher than value of 32.7 (Tangendjaja and Gunawan, 1988). This is could be affected by good quality of corn straw especially CP content. Eventhough NDF concentration of rice bran was lower than potato straw, DM degradability of potato straw was higher (P<0.01) than rice bran. It could be due to high lignin and silica contents in rice straw. Ibrahim (1995) reported that *in vitro* OM digestibility of rice bran was low caused by high lignin and silica contents. Leng (1990) defined that low-quality grass is indicated by low digestible value (< 55%) and low CP concentration (< 8%). Based on these criteria, sago waste, rice straw and cocoa pod are classified into low-quality feedstuffs.

Gas produced in *in vitro* experiment as result of substrate fermentation by microbe (CO_2 + CH_4) and buffer bicarbonate during fermentation (CO_2) (Getachew *et al.*, 1998). On average gas produced at 24 h of incubation was 87% of total gas produced by 48 h of incubation. This indicates that gas production rate decreased with increasing incubation time due to reducing substrate for fermentation process. Getachew *et al.* (2005) reported that gas produced at 24 h of incubation averaged 89% of total gas produces at 48 h. Gas production in soybean curd waste and sago waste at 24 and 48 h was higher (P<0.01) than other feeds, and the lowest was found in rice straw (Table 2). Gas production of each

Table 2. Gas Production and Coefficient of Nutrient Degradability of Feedstuffs after 24 and 48 h of incubation

Feedstuffs	Gas (ml/g DM)		Degradability (%)	
	24 h	48 h	DM	OM
Corn straw	150.6[bc]	173.6[c]	56.9[bc]	59.9[c]
Potato straw	180.9[b]	209.0[b]	62.4[b]	65.7[b]
Rice straw	134.3[c]	157.2[c]	27.8[e]	31.0[e]
Cocoa pod	162.4[bc]	186.5[bc]	46.2[d]	50.0[d]
Sago waste	220.0[a]	253.4[a]	58.3[bc]	60.9[bc]
Rice bran	151.1[bc]	179.3[bc]	52.8[c]	56.9[c]
Soybean curd residue	249.2[a]	274.6[a]	74.0[a]	78.7[a]
S.E.	7.70	7.31	1.28	1.23
P	<0.01	<0.01	<0.01	<0.01

DM: dry matter; OM: organic matter; SE: standard of error

Means with a column with different letters are significantly different (P<0.01)

feedstuff gets a long with OM degradability, as showed by correlation coefficient (r) between these variables was 0.75. In contrast, gas production was negative correlated with NDF content (r = 0.75). This result was in agreement with Getachew *et al.* (1998) result with value was -0.24. Rice straw had the lowest gas production among 7 feedstuffs tested in this study. However, gas production in rice straw was lower than reported by Wang *et al.* (2006) that gas production at 24 and 48 h of incubation were 35 and 44.2 ml/200 mg of substrate, respectively. The different in both results could be caused by differences in rice straw quality and rumen liquor source in in vitro experiment. There was positive correlation between CP content and gas production at 48 h of incubation and this result was consistent with result of Ndlovu and Nherera (1997) and Larbi *et al.* (1998) that r value was 0.16 and 0.39 respectively.

The CH_4 is produced by reduction of CO_2 and H_2 which catalyzed by enzyme secreted by methanogen. The CO_2 and H_2 are mainly produced during fermentation of structural carbohydrate i.e. hemicellulose (Takahashi, 2001). Methane production (ml/g DM) in rice straw, corn straw and cocoa pod was higher (P<0.01) as compared to soybean curd waste. High CH_4 production in those feedstuffs could be due high NDF content. The relationship between CH_4 production and NDF concentration was indicated with positive coefficient of correlation (r = 0.60; P<0.01). In contrast, NFC content had negative correlation with CH_4 production (r = -0.34). Santoso *et al.* (2007) concluded that there was strong correlation between CH_4 production (g/day) and NDF digested (g/day) (R^2=0.88). In addition, Moss *et al.* (2002) revealed that CH_4 production had positive correlated with NDF content (R^2=79%) and negative correlated with CP content (R^2=-76.8%). Average percentage of CH_4 from total gas production was 19%, which the highest was observed in rice straw and the lowest in soybean curd waste. In in vitro study, Getachew *et al.* (2005) reported that proportion CH_4 of the total gas was 16%, whereas Thalib (2008) found 22.5% CH_4 production.

Concentration NH_3-N in soybean curd waste was higher (P<0.01) as compared to other feedstuff, and the lowest was observed in sago waste. Concentration of N-NH_3 in both feedstuffs was consistent with crude protein content. This result was supported by strong correlation between CP content and NH_3-N as indicated with r = 0.83 (Table 4). Among 7 feedstuffs tested, concentration NH_3-N in sago waste was below normal range (7 mg N/100 ml) that recommended for maximum microbial N synthesis (Okorie *et al.*, 1977). Based on this result, it is recommended to combine sago waste with other

Table 3. Concentrations of NH_3-N (mg/100 ml), Total and Individual VFA (mmol/l) and Acetate : Propionate ratio in Supernatant after 48 h of incubation

Feedstuffs	NH_3-N	TVFA	C2	C3	C4	C2:C3
Corn straw	23.0^{b}	88.2^{b}	68.1^{b}	13.0^{c}	7.1^{abc}	5.2^{a}
Potato straw	12.4^{bc}	78.9^{b}	59.0^{bc}	13.8^{c}	6.1^{bcd}	4.3^{ab}
Rice straw	8.9^{c}	54.6^{c}	39.4^{d}	10.9^{c}	4.2^{d}	3.7^{b}
Cocoa pod	13.1^{bc}	80.1^{b}	63.2^{bc}	11.9^{c}	5.1^{cd}	5.4^{a}
Sago waste	4.8^{c}	79.2^{b}	52.5^{cd}	17.5^{b}	9.3^{a}	3.0^{b}
Rice bran	13.6^{bc}	67.6^{cb}	47.8^{cd}	11.9^{c}	7.8^{abc}	4.0^{ab}
Soybean curd residue	38.5^{a}	117.0^{a}	88.2^{a}	20.4^{a}	8.4^{ab}	4.3^{ab}
S.E	2.73	4.48	0.59	0.63	3.84	0.33
P	<0.01	0.01	<0.01	<0.01	<0.01	<0.01

TVFA: Total VFA; C2 : acetate; C3: propionate; C4: butirate; SE: standard of error
Means with a column with different letters different are significantly (P<0.01)

Table 4. Coefficient of Correlation (*r*) between Chemical Composition (%) and OM Degradability, Fermentation Characteristics, CH_4 Production

	CP	NDF	NFC
OMD (%)	0.67**	-0.75**	0.45
Gas 48 jam (ml/g DM)	0.32	-0.75**	0.61**
CH_4 48 jam (ml/g DM)	-0.53**	0.60**	-0.34
NH_3-N (mg/100 ml)	0.83**	-0.19	-0.20
Total VFA (mmol/l)	0.61**	-0.49*	0.23

*P<0,05; ** P<0,01
CP: crude protein; NDF: neutral detergent fiber; NFC: non-fiber carbohydrate; OMD: organic matter degradability; NH_3-N: ammonia N; VFA: volatile fatty acids

feedstuff which is had high CP content. Total VFA concentration in soybean curd waste was higher (P<0.01) than other feedstuff and the lowest was found in rice straw. Total VFA concentration in those feedstuffs was supported by OM degradability. The same pattern was also shown in acetate and propionate concentrations in soybean curd waste were higher than other feedstuffs. The ratio of acetate (C2) to propionate (C3) in cocoa pod and corn straw was higher than other feedstuffs. This indicates that high proportion of the potential NDF digested in both feedstuffs.

CONSLUSION

Feedstuffs from agricultural and food industry by-products varied greatly in their CP, NDF and NFC contents. Soybean curd waste had the best nutritive value as compared to other feedstuffs, as indicated by high CP content, and DM and OM degradability. There was positive correlation (*r*=0.60) between NDF content and CH_4 production (ml/g DM). However, NDF content was negative correlated (*r* = 0.75) with gas production (ml/g DM). Rice straw had the highest CH_4 production and sago waste had the lowest CH_4 production. The CH_4 production of feedstuff is influenced by NDF content.

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REFERENCES

Crowder, L.V. and H.R. Chheda. 1982. Tropical Grassland Husbandry. Longman Group Limited, New York, USA.

Estermann, B.L., F. Sutter, P.O. Schlegel, D. Erdin, H.R. Wettstein and M. Kreuzer. 2002. Effect of calf age and dam breed on intake, energy expenditure, and excretion of nitrogen, phosphorus, and methane of beef cows with calves. J. Anim. Sci. 80:1124-1134

Getachew, G., M. Blümmel, H.P.S. Makkar and K. Becker. 1998. *In vitro* gas measuring techniques for assessment of nutritional quality of feeds: a review. Anim. Feed Sci. Technol. 72:261-281

Getachew, G., P.H. Robinson, E.J. DePeters, S.J. Taylor, D.D. Gisi, G.E. Higginbotham and T.J. Riodan. 2005. Methane production from commercial dairy ration estimated using an *in vitro* gas technique. Anim. Feed Sci. Technol. 123-124:391–402

Harris, L.E. 1970. Nutrition Research Techniques for Domestic and Wild Animals. Vol. 1. Utah State University, Logan, Utah.

Ibrahim, M.N.M., S. Tamminga and G. Zemmelink. 1995. Degradation of tropical roughages and concentrate feeds in the rumen. Anim. Feed Sci. Technol. 54:81-92

Kondo, M., K. Kita and H. Yokota. 2006. Evaluation of fermentation characteristics and nutritive value of green tea waste ensiled with byproducts mixture for ruminants. Asian-Aust. J. Anim. Sci. 19 (4):533-540

Kumoro, A.C., G.C. Ngoh, M. Hasan, C.H. Ong and E.C. Teoh. 2008. Conversion of fibrous sago (*Metroxylon sagu*) waste into fermentable sugar via acid and enzymatic hydrolysis. Asian J. Sci. Res. 1(4):412-420

Kurihara, M.T. Magner, R.A. Hunter and G.J. McCrabb. 1999. Methane production and energy partition of cattle in the tropics. Br. J. Nutr. 81:227-234

Larbi, A., J.W. Smith, I.O. Kurdi, A.M. Raji and D.O. Ladipo. 1998. Chemical composition, rumen degradation, and gas production characteristics of some multipurpose fodder trees and shrubs during wet and dry seasons in the humid tropics. Anim. Feed Sci. Technol. 72:81-96

Leng, R.A. 1990. Factors affecting the utilization of poor-quality forages by ruminants particularly under tropical conditions. Nutr. Res. Rev. 3:277-303

Liu, J.X.. A. Susenbeth and K.H. Subekum. 2002. *In vitro* gas production measurements to evaluate interactions between untreated and chemically treated rice straw, grass hay, and mulberry leaves. J. Anim. Sci. 80:517-524

Minson, D.J. 1990. Forage in Ruminant Nutrition. Academic Press, San Diego, CA.

Menke, K.H. and H. Steingass. 1988. Estimation of the energetic feed value obtained from chemichal analysis and *in vitro* gas production using rumen fluid. Anim. Res. Dev. 28:7-55

Moss, A.R. and D.I. Givens. 2002. The effect of supplementing grass silage with soya bean meal on digestibility, *in sacco* degradability, rumen fermentation and methane production in sheep. Anim. Feed Sci. Technol. 97:127–143

Ndlovu, L.R. and F.V. Nherera. 1997. Chemical composition and relationship to *in vitro* gas production of Zimbabwean browsable indigenous tree species. Anim. Feed Sci. Technol. 69:121-129

Okorie, A.U., P.J. Buttery and D. Lewis. 1977. Ammonia concentration and protein synthesis in the rumen. Proc. Nutr. Soc. 36:38A.

Prasetyono, B.W.H.E., Suryahadi, T. Toharmat dan R. Syarief. 2007. Strategi Suplementasi Protein Ransum Sapi Potong Berbasis Jerami dan Dedak Padi. Med. Pet. 30(3):207-217

Utomo, R. 2004. Pengaruh Penggunaan Jerami Padi Fermentasi sebagai Bahan Dasar Pembuatan Pakan Komplit pada Kinerja Domba. Bul. Pet. 28(4):162-171

Santoso, B. dan B. Tj. Hariadi. 2008. Komposisi Kimia, Degradasi Nutrien dan Produksi Gas Metana *In vitro* Rumput Tropik yang Diawetkan dengan Metode Silase dan Hay. Med. Pet. 31(2):128-137

Santoso, B., B. Mwenya, C. Sar and J. Takahashi. 2007. Methane Production and Energy Partition in Sheep Fed Timothy Silage- or Hay-based Diets. JITV 12(1):27-33

Takahashi, J. 2001. Current state and issues of greenhouse gases emitted from animal agriculture in Japan. Proceedings of Greenhouse Gases and Animal Agriculture. Obihiro, Japan, November 7-11. P. 6-14

Tangendjaja, B. 1990. Pemanfaatan Limbah Padi untuk Pakan. Di dalam: Padi 3. (E. Soenaryo, D.S. Damardjati dan M. Syam, eds.). Badan Penelitian dan Pengambangan Pertanian, Pusat Penelitian dan Pengembangan Tanaman pangan, Bogor.

Tangendjaja, B. dan Gunawan. 1988. Jagung dan Limbahnya untuk Makanan Ternak. Badan Penelitian dan Pengambangan Pertanian, Pusat Penelitian dan Pengembangan Tanaman pangan, Bogor.

Thalib, A. 2008. Isolasi dan identifikasi bakteri asetogenik dari rumen rusa dan potensinya sebagai inhibitor metanogenesis. JITV. 13(3):197-2006

Tilley, J. M.A. and R.A. Terry. 1963. A two stage technique for the *in vitro* digestion of forage crops. J. Br. Grassland Soc. 18:104-111

Van Soest, P.J., J.B. Robertson and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597

Van Soest, P.J. 1994. Nutritional Ecology of The Ruminant. 2nd ed. Comstock Publishing Associates a Division of Cornell University Press, Ithaca and London.

Wang, H., Y. Wu, J. Liu and Q. Qian. 2006. Morphological fraction, chemical compositions and *in vitro* gas production of rice straw from wild and *brittle culm*1 variety harvested at different growth stages. Anim. Feed Sci. Technol.129:157-171

Wina. E., B. Tangendjaja and Dumaria. 2008. Effect of *Calliandra calothyrsus* on *in vitro* digestibility of soybean meal and tofu wastes. Livest. Res. Rural Dev. 20 (6). http://www.lrrd.org/lrrd20/6/wina20098.htm.